REMARKS

The Applicant respectfully requests reconsideration of pending claims 8-16.

Amendment to the Claims

Claim 8 is amended to clarify that the formation of embryoid bodies in the method occurs *in vitro*. As shown in the application at page 14, lines 4 – 20, the embryoid bodies may be formed in petri dishes after they are grown on mouse embryo fibroblasts, i.e., the embryoid bodies are formed *in vitro*.

Amendments to the Drawings

The Applicant offers an amendment to the sheet containing drawing Fig. 3B, which is attached, in order to correct two spelling errors appearing on the original figure. As amended, the Applicant submits the drawings are placed in proper form for allowance.

Objection to Claim 17

Claim 17 was objected to as being improperly dependent on claim 1. The Applicant requests withdrawal of claim 17 from current prosecution since the claim depends from an independent claim that is not currently elected for prosecution. The Applicant reserves the right to prosecute claim 17 in a follow-on divisional application.

Obviousness

The pending claims of the present invention are directed toward methods of directing differentiation of human embryonic cells to a specific cell type. The methods of independent claim 8, and dependent claims 9-16 therefrom, require the novel steps of (i) forming embryoid bodies (EBs) *in vitro* from human embryonic stem (ES) cells, and (ii) causing directed differentiation of human embryonic cells to a form a specific cell type. These novel steps are not taught by any of the cited art, alone or in combination.

Pending claims 8-16 stand rejected under 35 U.S.C. §103(a) as being obvious over various combinations of Thomson et al. (Science, Vol. 282, pp.1145-1147 (1998)); Keller (Curr. Opin. in Cell Biology, Vol. 7, pp. 862-869 (1995)); Wobus et al. (Cell Diff.,

Vol. 20, p. 81S (1987)); Vittet et al. (Blood, Vol. 88, pp. 3424-3431 (1996)); and Drab et al. (FASEB Journal, Vol. 11, pp. 905-915 (1997)).

Keller, Wobus, Vittet, and Drab are all directed toward aspects of *in vitro* differentiation of murine embryonic stem cells. None of the references suggest or teach the use of the revealed techniques as applied to human ES cells or human embryonic cells, as required by the claims.

Thomson teaches the production of undirected differentiated tumorous human ES cells *in vivo* by injecting human ES cells into SCID mice to form teratomas with all three germ layers, ectoderm, ectoderm, and mesoderm (See Thomson at 1146, column 1). Thomson, however, does not reveal either of the novel steps of claims 8-16 to form embryoid bodies from human ES cells, and to direct differentiation of human embryonic cells.

The Applicant maintains that claims 8-16 are patentable because the teachings of the cited art at the time of filing indicate no reasonable expectation of success in applying the murine ES cell techniques of Keller, Wobus, Vittet, and Drab to human ES cells as a step to cause directed differentiation of human embryonic cells.

The Cited Art Provides No Reasonable Expectation of Success

1. Mice and Humans are Different

a. Human Embryonic Cells Fail to Form Embryoid Bodies Using a Murine Embryoid Body Formation Technique

A significant difference exists between mice and humans with respect to their ES cells. For example, where mouse ES cells form EBs under a given set of conditions, human ES cells fail to form EBs. In fact, the conditions specified for mouse EB formation either kill the human ES cells, or, at best, show no consistent pattern of EB formation.

Reubinoff et al. (2000) reported that "manipulations used in our laboratory and elsewhere to facilitate embryoid body formation and multilineage differentiation of mouse ES cells induced death of human ES cells" (see reference AW in the IDS

submitted with the regular application on July 31, 2001, top of page 5 of IDS copy) (underlining added). Reubinoff et al. also stated that "[i]n these high-density cultures [of human embryonic stem cells], there was no consistent pattern of structural organization suggestive of the formation of embryoid bodies similar to those formed in mouse ES cell aggregates or arising sporadically in marmoset ES cell cultures" (see id.) (underlining added).

b. The Murine EB Formation Technique Also Fails with Primate ES Cells

The same conditions that fail to form EBs with human ES cells also fail when applied to other primate ES cells. In particular, the conditions failed to form EBs with rheusus monkey ES cells, and resulted in inconsistent and asychronous EB formation in marmoset ES cells.

In addition to the statements of Reubinoff et al., quoted earlier, Thomson and Marshall (1998) stated that "embryoid body formation by marmoset ES cells to date has been inconsistent and asynchronous" (see reference BK in the IDS submitted May 29, 2003, page 151, paragraph before section V) (underlining added). Itskovitz-Eldor et al. (2000) state that "[u]nlike murine ES cell lines, EBs formation from primate ES cells presented considerable difficulties", noting that "differentiation of ES cells from the rhesus monkey was disorganized, so that the resultant EBs failed to form vesicular structures" (see reference AY in the IDS submitted with the regular application on July 31, 2001, page 89, column 1, paragraph before *Materals and Methods* section) (underlining added). Indeed, Thomson and Marshall conclude that "culture conditions [need to] be established that allow efficient, synchronous development of organized primate embryoid bodies *in vitro* . . ." (see reference BK, page 151).

Since humans ES cells are more similar to other primate ES cells than murine ES cells, the inconsistency in forming embryoid bodies in primate ES cells points away from human embryonic cells forming embryoid bodies (See e.g., Thomson at 1147 column 1, stating that "[b]ecause of the similarities to humans and human ES cells, rhesus monkeys and rhesus ES cells provide an accurate model . . . for demonstrating the safety and efficacy of ES cell-base therapies).

c. Failure to Form EBs with Human/Primate ES Cells Points Away from a Reasonable Expectation of Success and Obviousness

MPEP §3143.03 states that "[a]t least some degree of predictability is required" to make a prima facie case of obviousness. Given the experimental failures to consistently form EBs with human, rhesus monkey, and marmoset ES cells using a murine EB formation technique, one skilled in the art had no reasonable expectation of success as of August 2000 that EBs could be formed consistently with human ES cells by combining Thomson with any combination of Keller, Wobus, Vittet, and Drab. Since EB formation from human ES cells is a required element of all the pending claims, the claims are both nonobvious and patentable.

2. Thomson Teaches Away from the Claimed Invention

Thomson not only fails to take one skilled in the art down the correct road toward practicing the claimed invention, but actually takes a skilled artisan down the wrong road entirely. While the pending claims require embryoid body formation, Thomson teaches teratoma formation; EBs are distinct from teratomas (See Reubinoff et al., who found that "[e]mbryoid bodies were not observed in the xenografts" of human ES cells in SCID mice," page 4 of reference AW). While the pending claims require the embryoid bodies to be formed *in vitro*, Thomson teaches an *in vivo* formation of the teratomas. While the pending claims require directed differentiation of the embryonic cells, Thomson teaches a teratoma composed of undirected, partially differentiated cells of all three germ layers (endoderm, ectoderm, and mesoderm); Thomson provides no evidence of directed differentiation. Thus, Thomson directs skilled artisans away from the required steps of the claimed invention, implying no reasonable expectation of success in the claimed methods.

3. Thomson with any of Keller, Wobus, Vittet, & Drab Provides No Reasonable Expectation of Success in Using Murine EB Formation Techniques on Human Embryonic Cells

Combining any of Keller, Wobus, Vittet, and Drab (herein referred to as "murine EB references") with Thomson provides no reasonable expectation of success in forming EBs from human ES cells because the techniques and results of their experiments are completely unrelated. First, the murine EB references teach embryoid body formation, while Thomson teaches teratoma formation. Second, the murine EB references only apply their techniques to mouse ES cells, while Thomson uses human ES cells. Third, the murine EB references teach *in vitro* formation of an embryoid body of murine ES cells, while Thomson teaches *in vivo* formation of a teratocarcinoma originating from injecting human ES cells into SCID mice. In short, these experiments are fundamentally different from each other; there is no reasonable expectation of success in combining their techniques and results.

Thus the prior art provides no reasonable expectation of success in forming embryoid bodies from human embryonic cells, and causing directed differentiation of said embryonic cells, as required by the pending claims. Therefore, for these and other reasons, claims 8 – 16 are patentable and nonobvious.

Conclusion

In view of the amendments and arguments presented, the Applicant respectfully requests allowance of pending claims 8-16.

Respectfully submitted,

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